Prolonged Elevation of Plasma Cortisol Levels in Dogs Treated with an 18 Amino Acid Synthetic Corticotrophin

Both native adrenocorticotrophin and synthetic corticotrophin preparations such as tetracosactide [β-corticotrophin-(1–24)-tetracosapeptide; Synacthen®] have a short duration of action, necessitating administration in adjuvants or repository vehicles. Recently, DESAULLES et al.[1] described the biological proterties of [D-ser[1], lys[17,18]]-β-corticotrophin-(1–18)-octadecapeptide amide (CIBA 41, 795–Ba). In addition to possessing enhanced potency, this synthetic polypeptide has a surprisingly long duration in rats[2] and human beings[3,4].

The dog has, in the past, been used extensively for studies of the effects of corticotrophins on adrenal corticosteroid production, utilizing adrenal vein cannulation techniques. However, until the recent advent of the protein binding and radioimmunoassays for steroids, it has been virutally impossible to measure the very low levels of glucocorticoids in the systemic circulation of this species. It was therefore of interest to compare the effects of 41,795-Ba with those of tetracosactide on peripheral plasma cortisol in the dog to determine if this species might be suitable for evaluating duration of action of synthetic corticotrophins.

41,795-Ba or tetracosactide were dissolved in saline and administered i.v. in doses of 0.3, 3.0 or 30 μg/kg to ovariectomized beagle dogs, weighing 7.5–10 kg each. The dogs were ovariectomized to eliminate a possible non-adrenal source of steroids might interfere with the assay for cortisol. Venous blood samples were obtained at 1, 2, 4 and 8 h, and in 1 experiment at 16 and 24 h post-injection. Plasma cortisol levels were determined by a slight modification of a competitive protein binding assay[5]. Cortisol served as the standard. Individual values were obtained by averaging 3–6 replicate analyses of each sample.

Both 41,795-Ba and tetracosactide increased plasma cortisol concentration at all doses tested (Table). However, plasma cortisol levels returned to the control range by the 4th h after injection of any dose of tetracosactide, whereas they remained elevated through the 4th and 8th h, respectively, after the 3 and 30 μg/kg doses of 41,795-Ba. These relationships are most clearly shown in the bottom third of the Table where average values are presented as percentages of the baseline control level for all dogs.

The mechanism whereby 41,795-Ba stimulates a prolonged secretion of cortisol in the dog is not known. Unlike retardant vehicles which mechanically slow the absorption of hormones from a s.c. injection depot the chemically modified corticotrophin is as effective or more effective when given i.v. as s.c. or i.m.[2,3].

[1] P. DESAULLES, B. RINIKER and W. RITTEL, in *Protein and Polypeptide Hormones* (Ed. M. MARGOULIES; Exerpta Medica Foundation Amsterdam, 1969), p. 489.

[2] R. MAIER, P. BARTHE, L. SCHENKEL-HULLIGER and P. DESAULLES, Acta endocr., Copenh. *68*, 458 (1971).

[3] J. KEENAN, J. THOMPSON, M. CHAMBERLAIN and G. BESSER, Br. med. J. *3*, 742 (1971).

[4] J. P. FELBER, M. AUBERT and R. DEGUILLAUME, Experientia *25*, 1196 (1969).

[5] E. JOHANSSON, Acta Endocr., Copenh. *61*, 592 (1969).

Plasma cortisol levels in female dogs administered Tetracosactide or 41,795-Ba

Treatment	Dose (mg/kg i.v.)	Dog No.	Time postinjection (h)						
			1	2	4	8	16	24	
Tetracosactide	0.3	1	106 ± 7	57 ± 5	13 ± 2	10 ± 1	—	—	Plasma cortisol[a] (ng/ml ± S.E.)
		2	99 ± 5	44 ± 4	7 ± 1	22 ± 2	—	—	
41,795-Ba	0.3	1	76 ± 6	42 ± 4	25 ± 1	13 ± 3	—	—	
		5	137 ± 5	108 ± 31	28 ± 4	4 ± 1	—	—	
		6	222 ± 4	163 ± 9	4 ± 3	12 ± 1	—	—	
Tetracosactide	3.0	3	134 ± 6	46 ± 2	9 ± 1	22 ± 1	—	—	
		4	94 ± 13	97 ± 11	4 ± 1	12 ± 2	—	—	
41,795-Ba	3.0	3	136 ± 11	147 ± 7	106 ± 12	46 ± 5	—	—	
		4	184 ± 9	170 ± 27	93 ± 2	23 ± 4	—	—	
Tetracosactide	30.0	5	115 ± 10	62 ± 4	9 ± 1	6 ± 1	—	—	
		6	160 ± 12	117 ± 12	9 ± 1	25 ± 2	—	—	
41,795-Ba	30.0	3	59 ± 3	78 ± 9	45 ± 3	79 ± 5	34 ± 3	20 ± 2	
		4	88 ± 12	64 ± 5	73 ± 9	84 ± 2	51 ± 4	30 ± 3	
		5	97 ± 7	105 ± 6	99 ± 3	95 ± 12	—	—	
		6	208 ± 11	227 ± 4	230 ± 9	172 ± 12	—	—	
	0.3	(1, 2)	397	196	38	62	—	—	Plasma cortisol (% of control value[a])
Tetracosactide	3.0	(3, 4)	443	277	26	66	—	—	
	30.0	(5, 6)	532	348	35	60	—	—	
	0.3	(1, 5, 6)	560	404	73	38	—	—	
41,795-Ba	3.0	(3, 4)	619	613	385	135	—	—	
	30.0	(3, 4, 5, 6)	438	460	433	416	165	58	

[a] The mean control plasma cortisol concentration for dogs 1–6 was 26 ± 8 ng/ml.

The data suggest that the dog is a suitable animal for evaluating duration of action of synthetic corticotrophins. Its size, large blood volume and ease of handling are also convenient for this type of study where serial samples are required. With as few as 2 dogs per group the difference in duration of action of any dose of tetracosactide and the two higher doses of 41,795-Ba was readily apparent. Furthermore, although within group variations in absolute cortisol levels obscured significant dose response relationships at any given time interval, dogs treated with 41,795-Ba showed clearcut dose-duration effects with time.

We conclude that 41,795-Ba stimulates a 2- to 4-fold more prolonged secretion of cortisol than does tetracosactide in the dog.

[6] Acknowledgements: We thank D. KARAFFA and J. CHINNERY for expert technical assistance.

Zusammenfassung. Die Bestimmung der Plasma-Konzentration von Cortisol in ovariectomierten Hunden, welche mit intravenösen Gaben von β-Corticotropin-oktadekapeptid (CIBA 41,795-Ba) oder Tetracosaktid vorbehandelt worden waren, ergab die interessante Tatsache, das in beiden Fällen die Cortisolkonzentration gleich stark erhöht war aber im ersten Fall die Erhöhung unter dem Einfluss von CIBA 41,795-Ba zwei- bis viermal länger erhalten blieb als nach Behandlung mit Tetracosaktid.

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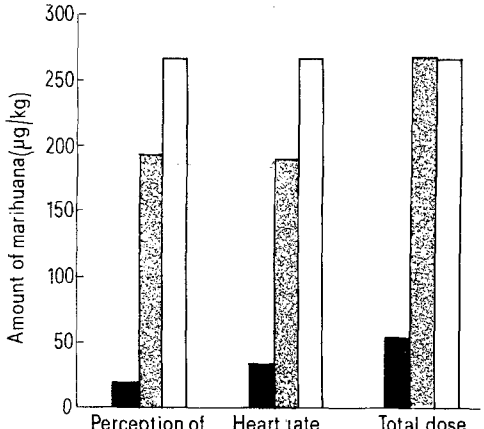
A Comparison of the Pharmacological Activity in Man of Intravenously Administered Δ^9-Tetrahydrocannabinol, Cannabinol, and Cannabidiol

The pharmacological activity of many of the chief constituents of marihuana have been systematically investigated in the Rhesus monkey by MECHOULAM et al.[1]. They found that Δ^9-tetrahydrocannabinol (Δ^9-THC) and Δ^8-tetrahydrocannabinol were active, and cannabinol, cannabidiol, and other cannabinoids present in the plant were not active. The development of a preparation to administer cannabinoids i.v. to man (PEREZ-REYES et al.[2]) has made it possible to make a comparison of the pharmacological activities and relative potencies of Δ^9-THC, cannabinol, and cannabidiol.

Method. 21 normal, paid, male volunteers were i.v. infused with Δ^9-THC, 6 with cannabinol, and 6 with cannabidiol. The preparation we have developed is a microsuspension of 10 mg of either of the cannabinoids in 50 ml of 25% salt-free human serum albumin. We have found that the amounts of cannabinoids can not be exceeded without inducing precipitation. Therefore, if different doses of these substances need to be infused, it is necessary to do so by changes in the rate of injection.



Drug		Perception of "high"	Heart rate acceleration	Total dose
Δ^9-THC $N=21$	mean	18.77	23.29	53.14
	S.D.	7.51	8.09	16.16
Cannabinol $N=6$	mean	194.41	189.59	269.56
	S.D.	41.11	103.01	39.78
Cannabidiol $N=6$	mean	>268.01	>268.01	268.01
	S.D.	–	–	22.38

Constituents injected i.v. to obtain certain specific effects. Since subjects varied in their body weight, all of the doses were calculated in terms of μg/kg of body weight. Figures indicated the mean values for the groups ± the standard deviation.

Subjects varied in their previous experience with marihuana from less than 1 cigarette/month to more than 5 cigarettes/week and were equally distributed in the groups. The subjects were hospitalized at the Clinical Research Unit of the North Carolina Memorial Hospital, Chapel Hill, N.C., and remained for 24 h until all the effects of the drugs had completely subsided. The heart rate was constantly recorded throughout the experiment by means of an Offner polygraph situated in a one-way screen room adjacent to the subjects' room.

To obtain the subjective evaluation of drug effects, that is of marihuana-like 'high', whether pleasant or unpleasant, we asked the subjects to rate themselves in a graph form provided for them. This rating was obtained at appropriate intervals for 6 h following drug administration. No specific instructions were given for these ratings, and each subject was free to utilize whatever criterion he wished for rating. We found that although there were variations in rating the magnitude of 'high', the pattern of the psychological experience in time was consistently similar.

Subjects were told that initially they would be i.v. infused with a drug-free solution (normal saline), and at some unspecified time, it would be replaced with the preparation containing either Δ^9-THC, cannabinol, or cannabidiol. The replacement of solutions without the subjects' awareness was possible because the Harvard constant infusion pump utilized for injection was located in the observation room. The subjects were instructed to report the moment they felt the action of the drug, that is the initial perception of marihuana-like effects, and to ask for the termination of the infusion as soon as they felt they had arrived at their desired level of 'high'. The volunteers were encouraged to receive the largest amount

[1] R. MECHOULAM, A. SHANI, H. EDERY and Y. GRUNFELD, *169*, 611 (1970).

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